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Original Research Article

Evaluation of Microbiological Safety of Organic and Conventionally Grown Rice

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ABSTRACT

The growth in popularity of organic foods has been driven, to a large extent, by claims that they are healthier than those grown by conventional farming methods. Yet, organic produce may also be susceptible to microbial contamination due to improper storage and hygienic conditions. Food borne micro organisms harbour themselves on the food material thereby influencing the food quality and consumer health. Amongst these microbes, the presence of Enterobacteriaceae family has been correlated with severe acute and systemic infections. Rice is a preferred cereal of India, especially Bengal. Therefore, the present study was carried out in order to determine the microbial safety of organic and conventional rice. A total of 12 samples grown in Kolkata, West Bengal and belonging to the above categories were analysed for total coliforms and the presence of members of the Enterobacteriaceae family. Most of the samples displayed a coliform count in the range of 10^3-10^5 CFU/ml, the highest being in the Mac Conkey agar. Microbial as well as biochemical determination indicated the inhabitance of enteric microorganisms especially *E.coli* in both conventional as well as organic rice varieties. The results therefore demonstrate a requirement of improved sanitation, hygienic conventions and storage of grains to ensure enhanced food safety.

Keywords: Conventional, Enterobacteriaceae, food safety, microbial contamination, organic

INTRODUCTION

Rice, also known as *Oryza sativa* is a plant belonging to the family of grasses, *Gramineae*. It is regarded as one of the three major crops in the world. It also forms a staple diet of one third of the world population. The global production of rice has been estimated to be at a level of 65 million tonnes. Asia is a leading rice producing nation accounting for 90% of the total rice production, 75% of which is consumed within the continent. This makes rice to be the most important food grain with respect to food security in Asia. [1] Moreover, it is considered as one of the most nutritious food source with carbohydrate, protein, zinc and niacin being the most important components. [2-3] Its immense diversity is also used for the treatment of indigestion, arthritis, paralysis, epilepsy and is also given to increase the strength during pregnancy and lactation. [4] However, it is also the crop that receives maximum quantity of fertilizers (40%) and pesticides (17-18%). Hence, the need of going organic has been important. [5-6] Organic agriculture system is a system that promotes environmentally, socially and economically sound foods. It is said that organic rice is the rice which is grown without the use of chemical pesticides and inorganic fertilizers. It however does not guarantee it to be free of contaminants. [7]

Despite being loaded with a number of health benefits, there are various types of food borne micro-flora (pathogenic or non-pathogenic) that adhere themselves to the food contents and surfaces for a long periods of time. These microorganisms play vital role in food degradation, toxification and pathogenicity of food products hence affecting food quality and safety. Amongst these microorganisms, Enterobacteriaceae family members have been known to cause serious health issues in humans. Its contamination in various food products especially in meat, milk and cereals has been a serious problem in tropical and subtropical areas due to favourable climatic conditions. [8] Surprisingly, The United States of America have reported 24-81 million food borne illnesses with an annual death of 9000 humans due to Enterobacteriaceae poisoning in the year 2011. [9] In India, the Centre for Science and Environment (CSE) had announced a red alert regarding the rise of food contamination. According to the report of 2013, *Escherichia coli, Salmonella sp* and *Campylobacter sp* were considered to be the major source of contaminants and were said to be multi drug resistant which in turn requires mass prevention from them. [10] Additionally, previous reports have correlated the presence of *Escherichia coli, Salmonella sp* and *Shigella sp* with severe infections. [11] The diseases are manifested either by ingestion of microbial pathogens or by the chemicals or bio-toxins produced by them. The intensity of disease can be accounted by the rate of mortality and morbidity outbreaks, considering the acute and chronic manifestations or severity that may lead to death, illness, health abnormalities and economic losses. [12] Furthermore, States like West Bengal, Nepal, New Delhi and Rajasthan in India in the past few years have witnessed similar outbreaks due to poor management of foods, consumption of adulterated or unpasteurised milk, ground meat, vegetables, cereal and water sources. [13] Rice being an excellent source of nutrients is considered as an ideal culture medium for the growth of Enterobacteriaceae family majorly due to fermentable carbohydrates and a favourable pH. Regrettably, organic produce of the same may be equally exposed to microbial contamination since organic fertilizers consists of manure which has been shown to harbour microorganisms of Enterobacteriaceae family. [Error! Reference source not found.]

Food borne illnesses have been a major concern in public health for decades with food handlers playing an important role in their transmission. Although there are various sources by which pathogens can contaminate food, multiply as well as cause infections in humans, yet the people who handle the food have been reported to be one of the major carriers. [14] Moreover, improper sanitation of surfaces and equipments may influence the burden of food borne microorganisms to a great extent. [16]

The present study enlightens the safety of the organic rice and conventionally grown rice because rice is one of the staple foods of India, especially Bengal. Rice is easily available and consumed by a large population of the country. This study attempts to add on to the limited researches carried out on the safety assessment of organic rice in order to enable careful selection of the same within the mass. Therefore, the study included a series of microbial analysis carried out in order to detect microbial contamination and understand the safety of organic and conventional rice.

METHODOLOGY

2.1. Selection of sample: Organic and conventionally grown rice samples of Kolkata, West Bengal were used for the study. Three types of commonly consumed rice viz; White rice, Brown Rice and Gobind Bhog Rice was collected. All the samples were sealed in zip locks and stored away from sunlight, air and water. Table 1 shows the variety and number of samples analyzed.

Table 1: Organic and conventional rice samples analyzed

Samples	Conventional (n)	Organic (n)
White Basmati Rice	2	2
Brown Rice	2	2
Gobind Bhog Rice	2	2
Total	6	6

n= number of sample analyzed

2.2. Sample preparation: The samples used for microbial analysis was prepared according to the methodology standardized by Taher A *et al.* [17] The samples were dissolved in peptone water. The media was sterilized using an autoclave at 121ºC. The sterilized mixture was grinded in a previously sterilized mortar pestle, until a uniform mixture was obtained. This was filled into sterilized test tubes and stored at refrigeration temperature.

2.3. Preparation of Microbiological Medias and Pour plating: Each prepared sample was stored in previously sterilized test tubes and was brought to room temperature before further analysis. The targeted Enterobacteriaceae family was enumerated in Nutrient Agar, Mac Conkey Agar, Eosin Methylene Blue Agar as well as Malachite Green Sucrose Agar by pour plating technique. The inoculated plates were incubated at 37°C for 24 hours to observe microbial growth. The differential media used were specific for the growth of *E. coli*, *Enterobacter sp* and *Salmonella sp* respectively. The results were expressed as Colony Forming Units (CFU/ml).

2.4. Fermentation Tests: For performing the Lactose as well as Sucrose fermentation, the test tubes were labelled appropriately. The lactose phenol broth and sucrose phenol broth were poured into the sterilized test tubes respectively. The sample was inoculated into the broth tubes by using Durham tubes. The media was mixed well and the broth was then incubated at 37°C for 24 hours to observe for colour change from red to yellow along with gas formation.

2.5. Gram Staining: For the purpose of staining, heat fixed bacterial smears was covered with crystal violet stain for 20-60 seconds and was washed with distilled water. The area was next covered with Gram's Iodine for 60 seconds and was washed with distilled water. The smear was decolourised with 95% ethyl alcohol or acetone for 10-20 seconds and was washed off using distilled water. The smear was covered with a counter stain, safranin for 30 seconds and washed off with water. It was then examined under the compound microscope under 45X magnification.

2.6. IMViC Tests: IMViC test is a series of four tests i.e. Indole, Methyl Red, Voges Prosekaeur, and Citrate Utilization tests which is majorly carried out for identification of the members of Enterobacteriaceae family. This is done in accordance with the change in colour of the medium upon addition of the respective indicators. The tests were conducted based on the methodology documented by Chholgyel N *et al.* [18] The appearance of red ring in Indole test gives a positive Indole reaction whereas the development of a stable red colour in MRVP broth indicated a positive MR test, ensuring the presence of *Escherichia coli*. Similarly the development of pink colour in VP broth represents a positive V-P test. Citrate positive organisms display growth and blue colour within the incubation period of citrate agar.

2.7. Statistical Analysis: Results were quantified as CFU/ml and expresses as mean±s.d. The significance of the analyzed data was calculated by standard student's t-test. Only $P<0.05$ was considered statistically significant.

RESULTS

3.1. Microbial counts in organic and conventional rice samples:

Micro organisms are a major cause of serious food borne illnesses which makes their identification equally important for the analysis of overall safety of a particular food commodity. Their presence above acceptable ranges can be hazardous to humans because they multiply at a constant rate under favourable conditions. Moreover, it majorly signifies improper handling and distribution channels.

The table 2 shows the mean count of microbes in all the four differential media which was conducted in order to interpret the safety of rice. Four different media were used, followed by a series of pour plating. Nutrient agar also known as the general purpose media was selected as the initial medium as it favours the growth of all kinds of microorganisms. This was followed by Mac Conkey agar specific for differentiating *E coli* and lactose fermenters; EMB agar facilitating the growth of Enterobacteriaceae family; and Malachite green sucrose agar prepared for enumeration of *Salmonella sp.*

Table 2: Microbial counts in organic and conventional rice samples

Sample	Safety Level (CFU/ml)	Microbial count on Nutrient Agar (%)		Microbial count on Mac Conkey Agar (%)		Microbial count on EMB Agar (%)		Microbial Count on Malachite green Agar (%)	
		Conventional	Organic	Conventional	Organic	Conventional	Organic	Conventional	Organic
White Basmati rice	$0\text{-}10^3$	68	60	70	78.4	82.4	70.6	80.92	96.6
	$10^4\text{-}10^5$	32	40	30	21.6	18.2	27.12	19.08	3.4
	$\geq 10^6$	0	0	0	0	0	0	0	0
Brown rice	$0\text{-}10^3$	0	52.7	88	44	36	58.4	28	24
	$10^4\text{-}10^5$	100	47.3	12	56	64	41.6	72	76
	$\geq 10^6$	0	0	0	0	0	0	0	0
Gobind Bhog rice	$0\text{-}10^3$	15	61	46.8	72	59	100	30	100
	$10^4\text{-}10^5$	85	39	53.2	28	41	0	70	0
	$\geq 10^6$	0	0	0	0	0	0	0	0

In accordance to table 2, the highest CFU was observed in conventional brown rice sample. The average CFU of conventional rice samples were found to be high compared to their organic counterparts. It was also noticed that the rice with greatest incidence of coliforms was brown rice, irrespective of the variety. Members of the Enterobacteriaceae family were identified by the colour and type of colonies formed on the differential media. As per the differential media used, the maximum growth was seen in Mac Conkey Agar specific for differentiating *Escherichia coli*, followed by Malachite green sucrose agar (specific for *Salmonella* sp) and EMB agar facilitating the growth of Enterobacteriaceae family. The type of colony formed was the criteria for the identification of the mentioned organisms. The average CFU/ml ranged between 10^3-10^5 CFU/ml. The presence of microbial contamination in organic and conventional rice samples could be associated with inadequate handling by vendors or improper storage facilities.

Increased prevalence of coliforms was seen in conventional rice samples when compared to the organic ones (Table 3). The mean microbial counts of the different organic and conventional rice varieties were compared to verify whether they differed significantly. Organic rice displayed the presence of the targeted microorganisms, although their counts were lower than their conventional counterparts. The colony counts were compared considering the cropping and food handling system. Outliers like climatic conditions and time of harvest was not included in the study.

Table 3: Microbial counts of total coliforms in different varieties of organic and conventional rice samples

Sample	Total Coliforms	
	Conventional	Organic
White Basmati Rice	3.09*±1.55	2.61±0.27
Brown Rice	3.24*±1.72	3.09*±0.86
Gobind Bhog Rice	2.94*±0.59	2.52±0.99

Result expressed as (mean±SD) log 10 CFU/ml
** P<0.05 considered as statistical significant*

3.2. Fermentation Tests: Fermentation test is the most common biochemical analysis used for detection of Enterobacteriaceae family, especially coliforms. The carbon sources lactose and sucrose upon fermentation yield acids that are subsequently detected by a colour changes in the medium, confirming the presence of microbial activity. Microbes also exhibit gas production as a result of fermentation, with varied end products like butyric acid, butyl alcohol, lactic acid, acetic acid, carbon dioxide etc. Table 4 clearly shows the ability of the bacteria to ferment both lactose and sucrose. Most of the samples changed the colour of the medium from red to yellow, indicating the tests to be positive and confirming the presence of lactose and sucrose fermenters respectively.

Furthermore, gas bubbles were also observed in the Durham's tubes which indicated gas production as the result of fermentation. On the other hand, presence of pink colour without gas bubbles indicated that the test was negative. Positive lactose and sucrose fermenters showed the presence of Enterobacteriaceae family, especially *Escherichia coli* whereas the negative test indicated Salmonella *sp* and *Enterobacter sp.*

Table 4: Fermentation analysis

Sample	Lactose Fermentation (%)				Sucrose Fermentation (%)			
	Conventional		Organic		Conventional		Organic	
	+	-	+	-	+	-	+	-
White Basmati rice	100	0	100	0	0	100	0	100
Brown rice	100	0	100	0	100	0	100	0
Gobind Bhog rice	50	50	100	0	0	100	0	100

3.3. Gram Staining: Gram staining forms one of the major tools for bacterial identification as well as classification. It helps in differentiating two major types of microbes viz.; gram positive and gram negative based on their cell wall composition. Table 5 shows that majority of the microorganisms stained pink showing that they were gram negative. These were a group of non spore forming, short rods and gram negative bacteria similar to the characteristics of Enterobacteriaceae family and coliforms. On the other hand, only a minor percentage was found to be gram positive. Therefore, the results display the inhabitance of rice majorly by gram negative bacteria thereby indicating coliform contamination.

Table 5: Gram staining

Sample	Gram Positive (%)		Gram Negative (%)	
	Conventional	Organic	Conventional	Organic
White Basmati rice	0	0	100	100
Brown rice	0	28	100	72
Gobind Bhog rice	22	0	78	100

3.4. IMViC analysis: Rice samples indicating the presence of the members of Enterobacteriaceae family in the former analysis were subjected to IMViC test for further confirmation. IMViC is a series of four tests i.e. Indole, Methyl Red, Voges Prosekaeur, and Citrate Utilization tests which is majorly carried out for identification of the members of Enterobacteriaceae family. This is done in accordance with the change in colour of the medium on addition of the respective indicators. Both organic and conventional rice samples confirmed the presence of Enterobacteriaceae family (Table 6), majorly *Escherichia coli* (as it gives positive indication with Indole and Methyl Red test and negative with VP and Citrate test).

Table 6: IMViC test

Sample	Indole Test (%)		Methyl Red Test (%)		Voges- Proskauer Test (%)		Citrate Utilization Test (%)	
	+	-	+	-	+	-	+	-
Organic white rice	100	0	100	0	0	100	0	100
Conventional white rice	100	0	100	0	0	100	0	100
Conventional Brown Rice	100	0	100	0	0	100	0	100
Conventional Gobind Bhog rice	100	0	100	0	0	100	0	100

DISCUSSION

The present study shows the presence of colonies in both the type of rice samples. The population level ranged between 10^3-10^5 CFU/ml. In accordance to the data obtained, the occurrence of the Enterobacteriaceae family was prominent as per the colonies obtained. However, among the various targeted species, presence of only *E.coli* was confirmed by the confirmatory tests. Highest incidence of contamination was seen in brown rice whereas the lowest was seen in the organic white rice. The mean counts of the microbial groups were also compared, regardless of the variety of rice. The prevalence of pathogens was found to be higher in conventional rice samples. Previous studies carried out with respect to microbial safety in rice in other areas also revealed similar results where the presence *E.coli, Salmonella sp, Enterobacter sp* as well as *Staphylococcus aureus* were found. [19] The factors which contribute to various food borne outbreaks like *Shigella sp, Klebsiella sp* and *Bacillus sp* were also confirmed in a few varieties. [20] Studies show that coliforms are a group of micro organisms which are widely spread in the environment is commonly seen in rice majorly due to cross contamination. [21] The presence of the microorganisms depend on many factors, including usage of fertilizer, irrigation water used as well as maintenance of good hygiene practices during handling. [22] Earlier reports on the microbial analysis of organic fertilizers reveal a lower microbial count in organic samples due to the thermophilic phase of composting and method of harvest. However, the pathogens might find its way into the produce after the phase due to the favourable nutrients, pH and water activity of the manure. [23-25] Although organic rice displayed lower levels of contamination compared to its conventional counterpart, yet the presence of coliforms were detected in both the varieties raising concerns of their microbiological safety. The study therefore highlights a requirement of stringent control measures. The results portray that the organic and conventional rice sold in the markets of Kolkata do contain considerable levels of coliforms indicating the need of adopting enhanced hygienic practices during farming, transport as well as processing. Moreover, awareness within the population regarding the sanitization of rice before consumption is necessary as it can minimize the risk of contamination as well as incidence of food borne diseases.

CONCLUSION

Rice has shaped the culture, diet and economy of thousands of people as it is considered as the staple food for more than 60% of the world population. Based on all the above tests, it was clearly observed that microorganisms were present in all samples of rice although with the highest count in conventional brown rice samples. All of them belonged to Enterobacteriaceae family, as they were gram negative, non-spore forming and sugar fermenters. The presence of microbes in rice could be associated with inadequate processing and handling by vendors, improper storage facilities, poor quality of grains and water used or cross contamination via the cooking utensils. This draws a conclusion that the food we consume cannot be theoretically claimed to be safe and nutritious unless scientific data is propagated for the same.

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